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L4: Entry 34 of 51 File: USPT Dec 31, 1996

US-PAT-NO: 5589337
DOCUMENT-IDENTIFIER: US 5589337 A

TITLE: Methods and diagnostic kits for determining toxicity utilizing bacterial stress promoters fused to reporter genes

DATE-ISSUED: December 31, 1996

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Farr; Spencer B.	Longmont	CO		

US-CL-CURRENT: 435/6; 435/29, 435/32

CLAIMS:

I claim:
1. A diagnostic kit for determining the toxicity of a compound or identifying an antitoxin to a toxic compound, said kit comprising:
a plurality of bacterial hosts, each of said hosts harboring a promoter which responds to stress, said promoter being operatively linked to a gene heterologous to said promoter and encoding an assayable product, wherein said plurality of hosts, in toto, comprise at least one promoter which responds to each of: redox stress, DNA stress, protein stress, energy stress and pH stress.
2. The diagnostic kit according to claim 1, wherein said promoter which responds to redox stress is sodA, soi28, katG, ahp, rdc, gsh, zwf or micF.
3. The diagnostic kit according to claim 1, wherein said promoter which responds to DNA stress is dinD, ada-alkA, ada, leu-500, gyr, top, mutT or nfo.
4. The diagnostic kit according to claim 1, wherein said promoter which responds to protein stress is rpoD, lon, clpB, merR, fepB-entC, groE or meto.
5. The diagnostic kit according to claim 1, wherein said promoter which responds to energy stress is sdh, cyo, cyd or unc.
6. The diagnostic kit according to claim 1, wherein said promoter which responds to pH stress is hag, micF, aniG or katF.
7. The diagnostic kit according to claim 1, wherein said plurality of bacterial hosts, in toto, comprises the promoters: soi28, dinD, hag, ada, gyr, katG, nfo, clpB, merR, top, cyd, micF, zwf, groE, katF and aniG.
8. The diagnostic kit according to claim 7, further comprising which is rdc, ahp, lon, unc, fepB-entC,

leu-500, cyo, sdh, rpoD, ada-alkA, sodA, mutT, gsh or meto.

9. The diagnostic kit according to any one of claims 1 to 8, wherein said gene encoding an assayable product is lacZ.

10. A method for determining the toxicity of a compound comprising the steps of:
(a) separately culturing each of a plurality of bacterial hosts, wherein each of said hosts harbors at least one promoter which responds to stress, said promoter being operatively linked to a gene heterologous to said promoter and encoding a detectable product, and wherein said plurality of hosts comprise, in toto, promoters which respond to each of: redox stress, DNA stress, protein stress, energy stress and pH stress;
(b) incubating each of said cultures with said compound;
(c) quantifying said detectable product in each culture; and
(d) creating a stress promoter induction profile for said compound.

11. The method according to claim 10, wherein said promoter which responds to redox stress is sodA, katG, ahp, soi28, rdc, gsh, micF or zwf.

12. The method according to claim 10, wherein said promoter which responds to DNA stress is dinD, ada-alkA, ada, leu-500, gyr, top, mutT or nfo.

13. The method according to claim 10, wherein said promoter which responds to protein stress is rpoD, lon, clpB, merR, fepB-entC, meto or groE.

14. The method according to claim 10, wherein said promoter which responds to energy stress is sdh, cyo, cyd or unc.

15. The method according to claim 10, wherein said promoter which responds to pH stress is hag, katF, micF or aniG.

16. The method according to claim 10, wherein said plurality of bacterial hosts, in toto, comprises the promoters: soi28, dinD, hag, ada, gyr, katG, nfo, clpB, merR, top, cyd, micF, zwf, groE, katF and aniG.

17. The method according to claim 16, wherein said plurality of bacterial hosts further comprises which is rdc, ahp, lon, unc, fepB-entC, leu-500, cyo, sdh, rpoD, ada-alkA, sodA, mutT, gsh or meto.

18. The method according to any one of claims 10 to 17, comprising the additional step of incubating said compound with an S9 liver extract, prior to step (b).

19. The method according to claim 18, wherein said gene encoding a detectable product is lacZ.

20. A method of identifying an antitoxin to a toxic compound comprising the steps of:
(a) determining the type of stresses caused by said toxic compound by the process according to claim 18;
(b) identifying a known toxic compound which, in the process according to claim 18, causes similar stresses to the stresses caused by said toxic compound; and
(c) identifying an antitoxin to said known toxic compound.

21. The method according to any one of claims 10 to 17, wherein said gene encoding a detectable product is lacZ.

22. A method of identifying an antitoxin to a toxic compound comprising the steps of:
(a) determining the type of stresses caused by said toxic compound by the process according to claim 19;
(b) identifying a known toxic compound which, in the process according to claim 19, causes similar stresses to the stresses caused by said toxic compound; and
(c) identifying an antitoxin to said known toxic compound.

23. A method of identifying an antitoxin to a toxic compound comprising the steps of:
(a) determining the type of stresses caused by said toxic compound by the process according to any one of claims 10-17;
(b) identifying a known toxic compound which, in the process according to any one of claims 10-17, causes similar stresses to the stresses caused by said toxic compound; and
(c) identifying an antitoxin to said known toxic compound.

L4: Entry 35 of 51 File: USPT Dec 17, 1996

US-PAT-NO: 5585232
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TITLE: Methods and diagnostic kits for determining toxicity utilizing E. coli stress promoters fused to reporter genes

DATE-ISSUED: December 17, 1996

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Farr; Spencer B.	Placitas	NM		

US-CL-CURRENT: 435/6; 435/252.33, 435/29

CLAIMS:

I claim:
1. A diagnostic kit for determining if a compound is a potential toxic, providing information about the mechanism of action of a toxin compound or identifying an antitoxin to a toxic compound, via the induction of repression of at least one stress gene promoter, said kit comprising:
(a) at least one E. coli host harboring a promoter which responds to redox stress;
(b) at least one E. coli host harboring a promoter which responds to DNA stress;
(c) at least one E. coli host harboring a promoter which responds to protein stress;
(d) at least one E. coli host harboring a promoter which responds to energy stress;
(e) at least one E. coli host harboring a promoter which responds to pH stress, each of said promoters being operatively linked to a gene encoding an assayable product, said gene being heterologous to the promoter to which it is operatively linked, and
(f) means for quantitating said assayable product.
2. The diagnostic kit according to claim 1, wherein said promoter which responds to redox stress is selected from sodA, katG, ahp, soi28, rdc or gsh.
3. The diagnostic kit according to claim 1, wherein said promoter which responds to DNA stress is selected from dinD, ada-alkA, leu-500, gyr, top, mutT or nfo.
4. The diagnostic kit according to claim 1, wherein said promoter which responds to protein stress is selected from rpoD, lon, clpB, merR, fepB-entC or meto.
5. The diagnostic kit according to claim 1, wherein said promoter which responds to energy stress is selected from sdh, cyo or unc.
6. The diagnostic kit according to claim 1, wherein said promoter which responds to pH stress is

selected from hag or katF.
7. The diagnostic kit according to claim 1, wherein said E. coli hosts, in toto, harbor at least the promoters: sdh, sodA, soi28, dinD, rpoD, hag, ada-alkA, gyr, katG, nfo, clpB, merR, unc, fepB-entC, top and gsh.
8. The diagnostic kit according to claim 7, wherein said E. coli hosts: in toro, additionally harbor at least one promoter selected from katF, rdc, lon, leu-500, cyo, mutt or meto.
9. The diagnostic kit according to any one of claims 1 to 8, wherein said gene encoding an assayable product is lacZ.
10. A method for determining if a compound is a potential toxin, or characterizing the mechanism of action of a toxic compound, via the induction or repression of at least one stress gene promoter, comprising the steps of:
(a) separately culturing each of:
(i) at least one E. coli host harboring a promoter which responds to redox stress;
(ii) at least one E. coli host harboring a promoter which responds to DNA stress;
(iii) at least one E. coli host harboring a promoter which responds to protein stress;
(iv) at least one E. coli host harboring a promoter which responds to energy stress; and
(v) at least one E. coli host harboring a promoter which responds to pH stress, each of said promoters being operatively linked to a gens encoding a detectable product, said gens being heterologous to the promoter to which it is operatively linked;
(b) incubating each of said cultures with said compound;
(c) quantifying said detectable product in each culture; and
(d) creating a stress promoter induction profile for said compound.
11. The method according to claim 10, wherein said promoter which responds to redox stress is selected from sodA, katG, ahp, soi28, rdc or gsh.
12. The method according to claim 10, wherein said promoter which responds to DNA stress is selected from dinD, ada-alkA, leu-500, gyr, top, mutT or nfo.
13. The method according to claim 10, wherein said promoter which responds to protein stress is selected from rpoD, lon, clpB, merR, fepB-entC or meto.
14. The method according to claim 10, wherein said promoter which responds to energy stress is selected from sdh, cyo or unc.
15. The method according to claim 10, wherein said promoter which responds to pH stress is selected from hag or katF.
16. The method according to claim 10, wherein said E. coli hosts, in toto, harbor at least the promoters: sdh, sodA, soi28, dinD, rpoD, hag, ada-alkA, gyr, katG, nfo, clpB, merR, unc, fepB-entC, top and gsh.
17. The method according to claim 16, wherein said E. coil hosts, in toto, additionally harbor at least one promoter selected from katF, rdc, lon, cyo, leu-500, mutT or meto.
18. The method according to any one of claims 10 to 17, comprising the additional step of incubating said compound with an S9 liver extract, prior to step (b).
19. The method according to any one of claims 10 to 17, wherein said gene encoding a detectable product is lacZ.
20. A method of identifying an antitoxin to a toxic compound comprising the steps of;
(a) determining the type of stresses caused by said toxic compound by the process according to any one of claims 10-17;
(b) identifying a known toxic compound which, in the process according to any one of claims 10-17, causes similar stresses to the stresses caused by said toxic compound; and
(c) identifying an antitoxin to said known toxic compound.
21. The method according to claim 18, wherein said gene encoding a detectable product is lacZ.
22. A method of identifying an antitoxin to a toxic compound comprising the steps of:
(a) determining the type of stresses caused by said toxic compound by the process according to claim 18;

(b) identifying a known toxic compound which, in the process according to claim 18, causes similar stresses to the stresses caused by said toxic compound; and
(c) identifying an antitoxin to said known toxic compound.
23. The method according to claim 20, wherein said gene encoding a detectable product is lacZ.
24. The method according to claim 22, wherein said gene encoding a detectable product is lacZ.

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TITLE: Methods and diagnostic kits for determining toxicity utilizing bacterial stress promoters fused to reporter genes

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Farr; Spencer B.	Longmont	CO		

US-CL-CURRENT: 435/6; 435/29, 435/32

CLAIMS:

I claim:
1. A diagnostic kit for determining the toxicity of a compound or identifying an antitoxin to a toxic compound, said kit comprising:
a plurality of bacterial hosts, each of said hosts harboring a promoter which responds to stress, said promoter being operatively linked to a gene heterologous to said promoter and encoding an assayable product, wherein said plurality of hosts, in toto, comprise at least one promoter which responds to each of: redox stress, DNA stress, protein stress, energy stress and pH stress.
2. The diagnostic kit according to claim 1, wherein said promoter which responds to redox stress is sodA, soi28, katG, ahp, rdc, gsh, zwf or micF.
3. The diagnostic kit according to claim 1, wherein said promoter which responds to DNA stress is dinD, ada-alkA, ada, leu-500, gyr, top, mutT or nfo.
4. The diagnostic kit according to claim 1, wherein said promoter which responds to protein stress is rpoD, lon, clpB, merR, fepB-entC, groE or meto.
5. The diagnostic kit according to claim 1, wherein said promoter which responds to energy stress is sdh, cyo, cyd or unc.
6. The diagnostic kit according to claim 1, wherein said promoter which responds to pH stress is hag, micF, aniG or katF.
7. The diagnostic kit according to claim 1, wherein said plurality of bacterial hosts, in toto, comprises the promoters: soi28, dinD, hag, ada, gyr, katG, nfo, clpB, merR, top, cyd, micF, zwf, groE, katF and aniG.
8. The diagnostic kit according to claim 7, further comprising which is rdc, ahp, lon, unc, fepB-entC,

leu-500, cyo, sdh, rpoD, ada-alkA, sodA, mutT, gsh or meto.

9. The diagnostic kit according to any one of claims 1 to 8, wherein said gene encoding an assayable product is lacZ.

10. A method for determining the toxicity of a compound comprising the steps of:

(a) separately culturing each of a plurality of bacterial hosts, wherein each of said hosts harbors at least one promoter which responds to stress, said promoter being operatively linked to a gene heterologous to said promoter and encoding a detectable product, and wherein said plurality of hosts comprise, in toto, promoters which respond to each of: redox stress, DNA stress, protein stress, energy stress and pH stress;

(b) incubating each of said cultures with said compound;

(c) quantifying said detectable product in each culture; and

(d) creating a stress promoter induction profile for said compound.

11. The method according to claim 10, wherein said promoter which responds to redox stress is sodA, katG, ahp, soi28, rdc, gsh, micF or zwf.

12. The method according to claim 10, wherein said promoter which responds to DNA stress is dinD, ada-alkA, ada, leu-500, gyr, top, mutT or nfo.

13. The method according to claim 10, wherein said promoter which responds to protein stress is rpoD, lon, clpB, merR, fepB-entC, meto or groE.

14. The method according to claim 10, wherein said promoter which responds to energy stress is sdh, cyo, cyd or unc.

15. The method according to claim 10, wherein said promoter which responds to pH stress is hag, katF, micF or aniG.

16. The method according to claim 10, wherein said plurality of bacterial hosts, in toto, comprises the promoters: soi28, dinD, hag, ada, gyr, katG, nfo, clpB, merR, top, cyd, micF, zwf, groE, katF and aniG.

17. The method according to claim 16, wherein said plurality of bacterial hosts further comprises which is rdc, ahp, lon, unc, fepB-entC, leu-500, cyo, sdh, rpoD, ada-alkA, sodA, mutT, gsh or meto.

18. The method according to any one of claims 10 to 17, comprising the additional step of incubating said compound with an S9 liver extract, prior to step (b).

19. The method according to claim 18, wherein said gene encoding a detectable product is lacZ.

20. A method of identifying an antitoxin to a toxic compound comprising the steps of:

(a) determining the type of stresses caused by said toxic compound by the process according to claim 18;

(b) identifying a known toxic compound which, in the process according to claim 18, causes similar stresses to the stresses caused by said toxic compound; and

(c) identifying an antitoxin to said known toxic compound.

21. The method according to any one of claims 10 to 17, wherein said gene encoding a detectable product is lacZ.

22. A method of identifying an antitoxin to a toxic compound comprising the steps of:

(a) determining the type of stresses caused by said toxic compound by the process according to claim 19;

(b) identifying a known toxic compound which, in the process according to claim 19, causes similar stresses to the stresses caused by said toxic compound; and

(c) identifying an antitoxin to said known toxic compound.

23. A method of identifying an antitoxin to a toxic compound comprising the steps of:

(a) determining the type of stresses caused by said toxic compound by the process according to any one of claims 10-17;

(b) identifying a known toxic compound which, in the process according to any one of claims 10-17, causes similar stresses to the stresses caused by said toxic compound; and

(c) identifying an antitoxin to said known toxic compound.

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NAME	CITY	STATE	ZIP CODE	COUNTRY
Farr; Spencer B.	Placitas	NM		

US-CL-CURRENT: 435/6; 435/252.33, 435/29

CLAIMS:

I claim:
1. A diagnostic kit for determining if a compound is a potential toxic, providing information about the mechanism of action of a toxin compound or identifying an antitoxin to a toxic compound, via the induction of repression of at least one stress gene promoter, said kit comprising:
(a) at least one E. coli host harboring a promoter which responds to redox stress;
(b) at least one E. coli host harboring a promoter which responds to DNA stress;
(c) at least one E. coli host harboring a promoter which responds to protein stress;
(d) at least one E. coli host harboring a promoter which responds to energy stress;
(e) at least one E. coli host harboring a promoter which responds to pH stress, each of said promoters being operatively linked to a gene encoding an assayable product, said gene being heterologous to the promoter to which it is operatively linked, and
(f) means for quantitating said assayable product.
2. The diagnostic kit according to claim 1, wherein said promoter which responds to redox stress is selected from sodA, katG, ahp, soi28, rdc or gsh.
3. The diagnostic kit according to claim 1, wherein said promoter which responds to DNA stress is selected from dinD, ada-alkA, leu-500, gyr, top, mutT or nfo.
4. The diagnostic kit according to claim 1, wherein said promoter which responds to protein stress is selected from rpoD, lon, clpB, merR, fepB-entC or meto.
5. The diagnostic kit according to claim 1, wherein said promoter which responds to energy stress is selected from sdh, cyo or unc.
6. The diagnostic kit according to claim 1, wherein said promoter which responds to pH stress is

selected from hag or katF.
7. The diagnostic kit according to claim 1, wherein said E. coli hosts, in toto, harbor at least the promoters: sdh, sodA, soi28, dinD, rpoD, hag, ada-alkA, gyr, katG, nfo, clpB, merR, unc, fepB-entC, top and gsh.
8. The diagnostic kit according to claim 7, wherein said E. coli hosts: in toro, additionally harbor at least one promoter selected from katF, rdc, lon, leu-500, cyo, mutt or meto.
9. The diagnostic kit according to any one of claims 1 to 8, wherein said gene encoding an assayable product is lacZ.
10. A method for determining if a compound is a potential toxin, or characterizing the mechanism of action of a toxic compound, via the induction or repression of at least one stress gene promoter, comprising the steps of:
(a) separately culturing each of:
(i) at least one E. coli host harboring a promoter which responds to redox stress;
(ii) at least one E. coli host harboring a promoter which responds to DNA stress;
(iii) at least one E. coli host harboring a promoter which responds to protein stress;
(iv) at least one E. coli host harboring a promoter which responds to energy stress; and
(v) at least one E. coli host harboring a promoter which responds to pH stress, each of said promoters being operatively linked to a gens encoding a detectable product, said gens being heterologous to the promoter to which it is operatively linked;
(b) incubating each of said cultures with said compound;
(c) quantifying said detectable product in each culture; and
(d) creating a stress promoter induction profile for said compound.
11. The method according to claim 10, wherein said promoter which responds to redox stress is selected from sodA, katG, ahp, soi28, rdc or gsh.
12. The method according to claim 10, wherein said promoter which responds to DNA stress is selected from dinD, ada-alkA, leu-500, gyr, top, mutT or nfo.
13. The method according to claim 10, wherein said promoter which responds to protein stress is selected from rpoD, lon, clpB, merR, fepB-entC or meto.
14. The method according to claim 10, wherein said promoter which responds to energy stress is selected from sdh, cyo or unc.
15. The method according to claim 10, wherein said promoter which responds to pH stress is selected from hag or katF.
16. The method according to claim 10, wherein said E. coli hosts, in toto, harbor at least the promoters: sdh, sodA, soi28, dinD, rpoD, hag, ada-alkA, gyr, katG, nfo, clpB, merR, unc, fepB-entC, top and gsh.
17. The method according to claim 16, wherein said E. coil hosts, in toto, additionally harbor at least one promoter selected from katF, rdc, lon, cyo, leu-500, mutT or meto.
18. The method according to any one of claims 10 to 17, comprising the additional step of incubating said compound with an S9 liver extract, prior to step (b).
19. The method according to any one of claims 10 to 17, wherein said gene encoding a detectable product is lacZ.
20. A method of identifying an antitoxin to a toxic compound comprising the steps of;
(a) determining the type of stresses caused by said toxic compound by the process according to any one of claims 10-17;
(b) identifying a known toxic compound which, in the process according to any one of claims 10-17, causes similar stresses to the stresses caused by said toxic compound; and
(c) identifying an antitoxin to said known toxic compound.
21. The method according to claim 18, wherein said gene encoding a detectable product is lacZ.
22. A method of identifying an antitoxin to a toxic compound comprising the steps of:
(a) determining the type of stresses caused by said toxic compound by the process according to claim 18;

(b) identifying a known toxic compound which, in the process according to claim 18, causes similar stresses to the stresses caused by said toxic compound; and
(c) identifying an antitoxin to said known toxic compound.
23. The method according to claim 20, wherein said gene encoding a detectable product is lacZ.
24. The method according to claim 22, wherein said gene encoding a detectable product is lacZ.